mass spectrometry analysis using the first mobile phase of 0.1% formic acid in water and a second mobile phase of was 0.1% formic acid in acetonitrile.

20. The method of claim **19**, wherein the percent of oxidized species of aflibercept in said flowthrough fraction are reduced by at least about 10% compared to the percent of oxidized species of aflibercept in said affinity eluate.

21. The method of claim **19**, wherein said oxidized amino acid residue is selected from the group consisting of methionine, tryptophan, histidine, phenylalanine, tyrosine and a combination thereof.

22. The method of claim **19**, wherein said oxidized amino acid residue is selected from an amino acid residue on a polypeptide having an amino acid sequence as set forth in the group consisting of: SEQ ID NO.: 17, SEQ ID NO.: 18, SEQ ID NO.: 19, SEQ ID NO.: 20, SEQ ID NO.: 21, SEQ ID NO.: 22, SEQ ID NO.: 23, and SEQ ID NO.: 67.

23. The method of claim **19**, wherein said protein further comprises one or more variant amino acid residues selected from a polypeptide having an amino acid sequence as set forth in the group consisting of: SEQ ID NO.: 17, SEQ ID NO.: 18, SEQ ID NO.: 19, SEQ ID NO.: 20, SEQ ID NO.: 21, SEQ ID NO.: 22, SEQ ID NO.: 23, SEQ ID NO.: 56, SEQ ID NO.: 64, SEQ ID NO.: 65, SEQ ID NO.: 66, SEQ ID NO.: 67, SEQ ID NO.: 68, SEQ ID NO.: 69, SEQ ID NO.: 70, SEQ ID NO.: 71, and combinations thereof.

24. A method of producing aflibercept from a clarified harvest of a cell cultured in a chemically defined medium (CDM), comprising:

(a) collecting a protein sample from a clarified harvest, wherein said protein sample comprises aflibercept and at least one aflibercept variant, and wherein said protein sample has a b* value of more than 0.5 when the concentration of said protein sample is normalized to 10.0 g/L;

(b) binding aflibercept from said clarified harvest of step (a) to a first capture chromatography and eluting said aflibercept; and

(c) subjecting said aflibercept from (b) to an anion exchange chromatography (AEX) column and collecting at least one flowthrough fraction, wherein said flowthrough fraction has a less intense yellow-brown color compared to said protein sample from said clarified harvest when concentration of protein in said flowthrough fraction is normalized to 10.0 g/L.

25. The method of claim **24**, wherein said capture chromatography comprises Protein A resin.

26. The method of claim **24**, wherein said clarified harvest comprises one or more aflibercept variants, wherein said variants have at least one oxidized amino acid residue selected from group consisting of methionine, tryptophan, histidine, phenylalanine, tyrosine and a combination thereof.

27. The method of claim **26**, wherein said oxidized amino acid residue is histidine.

28. The method of claim **26**, wherein said oxidized amino acid residue is tryptophan.

29. The method of claim **24**, wherein said aflibercept variant is selected from an amino acid residue on a polypeptide having an amino acid sequence as set forth in the group consisting of: SEQ ID NO.: 17, SEQ ID NO.: 18, SEQ ID NO.: 19, SEQ ID NO.: 20, SEQ ID NO.: 21, SEQ ID NO.: 22, SEQ ID NO.: 23, SEQ ID NO.: 56, SEQ ID NO.: 64, SEQ ID NO.: 65, SEQ ID NO.: 66, SEQ ID NO.: 67, SEQ ID NO.: 68, SEQ ID NO.: 69, SEQ ID NO.: 70, SEQ ID NO.: 71, and combinations thereof.

30. The method of claim **24**, further comprising after collecting aflibercept from said clarified harvest, subjecting aflibercept to one or more further chromatographic steps selected from the group consisting of: cation exchange chromatography, hydrophobic interactive chromatography, size exclusion chromatography and a combination thereof.

* * * * *